Data Evaluation Report on the Acute Toxicity of Rimon 10EC to Freshwater Invertebrates - *Daphnia magna*
PMRA Submission Number{.....} EPA MRID Number 45638413

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D285479
	OECD Data Point	
	EPA MRID	45638413
	EPA Guideline	§72-2

Test material: Rimon 10EC **Purity:** 9.1% (w:w) Novaluron
Common name: Novaluron (active ingredient)
Chemical name: IUPAC: 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea (a.i.)
CAS name: N-[[[3-Chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]carbonyl]-2,6-difluorobenzamide (a.i.)
CAS No.: 116714-46-6 (a.i.)
Synonyms: "RIMON" 10 EC

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CITATION: Jenkins, C.A. 1999. "RIMON" 10EC: Acute Toxicity to *Daphnia Magna*. Unpublished study performed by Huntingdon Life Sciences Ltd, Eye, Suffolk, England. Laboratory Project Identification No. MAK/463/983514. Study submitted by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel. Study initiated December 16, 1997 and completed January 15, 1999.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Rimon 10EC [a formulated product containing 9.1% (w:w) Novaluron] to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 0.156, 0.313, 0.625, 1.25, 2.5, 5, and 10 μg/L. For all but the highest test level, the measured values were below the accurate limit of quantification (10.1 μg/L); however, the stability of Rimon 10EC was established under test conditions.

After 48 hours, 5, 30, 55, and 85% immobilization were observed at the 1.25, 2.5, 5, and 10 μg/L levels; immobility at the 1.25 μg/L was not considered a significant treatment-related effect. The 48-hour EC_{50} (with 95% C.I.) was 4.31 (3.34-5.77) μg/L, which categorizes Rimon 10EC as very highly toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOEC and LOEC levels were 1.25 and 2.5 μg/L, respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as CORE.

Results Synopsis

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour

EC_{50}: 4.31 μg/L	95% C.I.: 3.34-5.77 μg/L
Slope: 2.91	95% C.I.: 1.93-3.89
NOEC: 1.25 μg/L	
LOEC: 2.5 μg/L	

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in Procedure 202 of the OECD Guidelines (1984); Annex to the European Commission Directive 92/69/EEC, Part C2 of the EC Methods for Determination of Ecotoxicity (1992); Series §72-2 of the U.S. EPA Pesticide Assessment Guidelines (1982); and Hazard Evaluation Division, Standard Evaluation Procedure (1985). Deviations from §72-2 included:

1. Pre-test health (including mortality) of the laboratory culture and/or brood was not described.
2. Feeding during the study was not specified.
3. The dilution water was treated laboratory tap water.
4. The pH range (7.7-7.9) was slightly higher than recommended (7.2-7.6).
5. The loading rate was not specified.
6. The 0.156, 0.313, and 0.625 μg/L treatments (three lowest test concentrations) were not sampled for analysis, and although the 1.25, 2.5, and 5 μg/L treatment were sampled, results were considered in

estimate since levels were below the accurate limit of quantification (LOQ, 10.1 μg/L). In addition, one result for the 10 μg/L level was also below the LOQ and considered in estimate.

These deviations do not affect the scientific validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards set forth by the United Kingdom (1997), EC Council Directive (1986), OECD (1997), U.S. EPA (1989), and JMAFF (1984).

A. MATERIALS:

1. Test Material Rimon 10EC (formulated product)

Description: Clear liquid

Lot No./Batch No. : 960917

Purity: 9.1% (w:w) Novaluron

Stability of Compound Under Test Conditions: Since the majority of concentrations were below the accurate limit of quantification (LOQ, 10.1 μg/L), some degree of variation was observed in the analytical results. However, it was apparent that Rimon 10 EC did not degrade in samples analyzed at 0 and 48 hours from the 1.25, 2.5, 5, and 10 μg/L test concentrations (Table 1, p. 21). Furthermore, analysis of the aqueous stock solution (100 μg/L) after 48 hours averaged 97% of the mean 0-hour concentration.

Storage conditions of test chemicals: Stored at 4°C in the dark.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna*

Age at test initiation: Juvenile, <24 hours old

Source: In-house laboratory cultures maintained since 1989 (original supplier: University of Sheffield, England).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Definitive test concentrations were based upon results of two 48-hour static range-finding tests (pp. 14 and 16). The test concentrations for the first and second range-finding tests were 0.1, 1, 10, or 100 μg/L and 0.01, 0.1, 1, 10, or 100 μg/L, respectively. In the first test, all of the daphnids exposed at 0.1 to 100 μg/L were immobilized after 48 hours. In the second test, the NOEC was 1 μg/L and the LOEC was 10 μg/L, with a 48-hour EC_{50} of 3.16 μg/L. The study author states the difference in results between the two tests was probably due to the timing of the animals' first molt, since the active ingredient of the product is a chitin inhibitor.

b) Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous laboratory cultures were maintained. Same as test *Daphnia* cultures were fed at least 5 times/week with unicellular green algae *Chlorella vulgaris* (2 to 8 x 10^5 cells/mL) and yeast (0.04 mg/L). Not specified	Feeding during the study was not specified. *EPA requires 7 day minimum acclimation period. No feeding during study.*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static N/A N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	No aeration was used during the study.	

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass with a glass lid 400 mL 200 mL	*EPA requires: size 20 mL or 3.9 L fill 200 mL*
Source of dilution water	Laboratory tap water was filtered through active carbon to remove chlorine and softened with reverse osmosis tap water.	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 49 mg $CaCO_3$/L 7.7-7.9 100% 20.0-20.2°C 0.8-0.9 mg/L Not specified See Appendix 2, p. 24 Not detected <0.01 mg/L	The pH was slightly higher than recommended. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 4 4	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 5 5	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	0 (negative control), 0.156, 0.313, 0.625, 1.25, 2.5, 5, and 10 µg/L <1 (control), 2.92, 3.68, 6.18, and 12.16 µg/L (the four highest test concentrations)	Mean-measured values were reviewer-calculated from 0 and 48 hour analytical results (Table 1, p. 21). The 0.156, 0.313, and 0.625 µg/L treatments were not sampled for analysis. Results from the 1.25, 2.5, and 5 µg/L treatments were considered estimates since the values were below the accurate limit of quantification (10.1 µg/L). *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	N/A	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark, with a transition period.	Light intensity for the test area was 371 lux. *EPA requires 16 hours light, 8 hours dark.*
Stability of chemical in the test system	The 48-hour analyzed concentrations were 68-132% of the 0-hour analyzed concentrations.	Since the majority of concentrations were below the accurate limit of quantification (LOQ, 10.1 µg/L), some degree of variation was observed in the analytical results. However, it was apparent that Rimon 10 EC did not degrade in samples analyzed at 0 and 48 hours from the 1.25, 2.5, 5, and 10 µg/L test concentrations (Table 1, p. 21). Furthermore, analysis of the aqueous stock solution (100 µg/L) after 48 hours averaged 97% of the mean 0-hour concentration.

Parameter	Details	Remarks *Criteria*
Recovery of chemical Level of Quantitation Level of Detection	Mean of 111% of nominal 10.1 μg/L 1 μg/L	Based on the analyzed stock solution (100 μg/L).
Positive control {if used, indicate the chemical and concentrations}	None concurrent with the study.	The sensitivity of juvenile daphnia cultured in this laboratory is periodically assessed using potassium dichromate (p. 9). The results for the most recent test performed prior to the definitive study indicated that the 48-hour EC_{50} was 0.18 mg/L and within the range typically obtained in this laboratory (of 0.05 to 0.3 mg/L).
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Numbers of mobile (submerged and floating) and immobile (submerged and floating) daphnids were recorded.	
Observation intervals	After 3, 24, and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. SUB-LETHAL TOXICITY ENDPOINTS:

Table 3: Sublethal Effect of Rimon 10EC on *Daphnia magna.*

Treatment, μg/L measured and (nominal conc.)	Observation period					
	3 hours		24 hours		48 hours	
	endpoint	% affected[1]	endpoint	% affected[1]	endpoint	% affected[1]
Dilution water Control	Immobile	0	Immobile	0	Immobile	0
(0.156)	Immobile	0	Immobile	0	Immobile	0
(0.313)	Immobile	0	Immobile	0	Immobile	0
(0.625)	Immobile	0	Immobile	0	Immobile	0
2.92 (1.25)	Immobile	0	Immobile	0	Immobile	5
3.68 (2.5)	Immobile	0	Immobile	0	Immobile	30
6.18 (5)	Immobile	0	Immobile	10	Immobile	55
12.16 (10)	Immobile	0	Immobile	40	Immobile	85
NOEC, μg/L*	Not determined		2.5		1.25	
LOEC, μg/L*	Not determined		5		2.5	
EC_{50} (with 95% C.I.), μg/L*	Not determined		Not determined		4.22 (3.22-5.79)	

[1] The % affected was reviewer-calculated from number of immobile daphnids.
* Study author's results are based on nominal values.

B. REPORTED STATISTICS:

The EC_{50} value (with 95% C.I.) after 48 hours was calculated using the moving average method via the computer program by Stephan (1977, 1982). The NOEC was visually determined based on the level that caused $\leq$ 10% immobilization. Nominal concentrations were used in all estimations.

C. VERIFICATION OF STATISTICAL RESULTS:

The NOEC and LOEC for immobilization was determined using Fisher's Exact Test via TOXSTAT statistical software. The EC_{50} (with 95% C.I.) was determined using the probit method. Since mean-measured values were not determined at all concentrations, and since the majority of those determined were below the LOQ, nominal concentrations were used in all estimations.

48-Hour

EC_{50}: 4.31 μg/L	95% C.I.: 3.34-5.77 μg/L
Slope: 2.91	95% C.I.: 1.93-3.89
NOEC: 1.25 μg/L	
LOEC: 2.5 μg/L	

D. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-2 that affected the acceptability of this study. Although mean-measured concentrations were not determined for the three lowest test concentrations, and the majority of those that were determined were below the accurate LOQ, it was apparent that Rimon 10EC did not degrade under actual use conditions. Since an accurate Toxicity Category could be assigned, the main objective of this guideline was fulfilled.

E. REVIEWER'S COMMENTS:

The reviewer's NOEC and LOEC values for immobilization were identical to the study author's. The reviewer's EC_{50} estimate provided a narrower 95% confidence interval than the study author's, so it is reported in the Executive Summary and Conclusions sections.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2, and is classified as CORE. Based on the results of this study, Rimon 10EC [a formulated product containing 9.1% (w:w) Novaluron] is categorized as very highly toxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour

EC_{50}: 4.31 μg/L	95% C.I.: 3.34-5.77 μg/L
Slope: 2.91	95% C.I.: 1.93-3.89
NOEC: 1.25 μg/L	
LOEC: 2.5 μg/L	

III. REFERENCES:

US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 72 Aquatic Organisms Testing §72-2 Acute toxicity test for freshwater aquatic invertebrates, October 1982.

US EPA Hazard Evaluation Division, Standard Evaluation Procedure: Acute toxicity test for freshwater invertebrates. EPA-540/9-85-006, June 1985.

OECD Guidelines for Testing of Chemicals. "*Daphnia*, sp., Acute Immobilisation Test and Reproduction Test, Part 1. Procedure 202", adopted 4 April 1984.

Official Journal of the European Communities. L383A. Part C: Methods for Determination of Ecotoxicity: C2. Acute toxicity for *Daphnia*. Vol 35, 29 December 1992. ISSN 0378-6978.

Stephan, C.E. 1977. Methods for calculating an LC_{50}. Aquatic Toxicology and Hazard Evaluation. ASTM STP 634.

Stephan, C.E. 1982. A computer program for calculating an LC_{50}. US Environmental Protection Agency.

Huntingdon Life Science Report No. MAK404/970470. "RIMON" Technical: Acute toxicity to *Daphnia magna.*

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

NOEC and LOEC

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.156	20	0	
2	0.313	20	0	
3	0.625	20	0	
4	**1.25**	**20**	**1**	
5	2.5	20	6	*
6	5	20	11	*
7	10	20	17	*

LC50

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.364424

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1198137	4.215168	3.217035	5.79379

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1132324	1	.9897122

SLOPE = 2.913439
95 PERCENT CONFIDENCE LIMITS = 1.933066 AND 3.893812

LC50 = 4.30801
95 PERCENT CONFIDENCE LIMITS = 3.340049 AND 5.769696

LC10 = 1.57894
95 PERCENT CONFIDENCE LIMITS = .9291528 AND 2.156119